RESEARCH PAPER

Chitosan-Coated Nanostructured Lipid Carriers for Enhanced Solubility and Oral Bioavailability of Azilsartan Medoxomil: *Ex Vivo* and *In Vivo* Pharmacokinetic Evaluation

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ABSTRACT

Azilsartan medoxomil (AZM) is an effective antihypertensive prodrug; however, its clinical performance is constrained by poor aqueous solubility and limited intestinal permeability, leading to inconsistent oral bioavailability. Lipid-based nanocarriers, particularly nanostructured lipid carriers (NLCs), have gained attention for improving oral delivery of hydrophobic drugs. Surface modification with chitosan is expected to further enhance intestinal interaction and drug absorption. AZM-loaded NLCs and chitosan-coated NLCs (AZM-CH-NLC) were developed and optimized using a design-of-experiments approach. The optimized formulation was evaluated through bioadhesion studies using a texture analyser, ex vivo intestinal permeation using the non-everted sac method, confocal laser scanning microscopy (CLSM) for tissue penetration, and in vivo pharmacokinetic analysis in Wistar rats. Pharmacokinetic parameters were determined using non-compartmental analysis. The AZM-CH-NLC formulation demonstrated significantly higher mucoadhesion compared to uncoated NLCs and drug suspension. Ex vivo studies revealed enhanced intestinal permeation, with increased flux and permeability coefficients. AZM-CH-NLC exhibited approximately two-fold higher flux compared to AZM-NLC and more than four-fold higher than the suspension. CLSM imaging confirmed deeper and more uniform penetration of coated nanoparticles within intestinal tissues. In vivo pharmacokinetic evaluation showed a marked increase in peak plasma concentration (Cmax) and overall exposure (AUC) for AZM-CH-NLC, indicating improved oral bioavailability. Pharamacokinetic parameters showed, AZM-CH-NLC achieved nearly two-fold higher Cmax compared to the suspension. The $AUC_{0–t}$ value was also substantially increased, indicating enhanced overall exposure. The improved performance of AZM-CH-NLC is attributed to the combined effects of nanoscale size, lipid matrix solubilization, and chitosan-mediated mucoadhesion, which prolong intestinal residence and facilitate drug transport across epithelial barriers. Chitosan-coated NLCs represent a novel framework for enhancing oral delivery of AZM, offering improved absorption and systemic exposure, with potential applicability to other poorly soluble drugs.

Keywords: Azilsartan medoxomil, Nanostructured Lipid Carriers (NLCs), Chitosan coating, Oral bioavailability, Intestinal permeation, Pharmacokinetics

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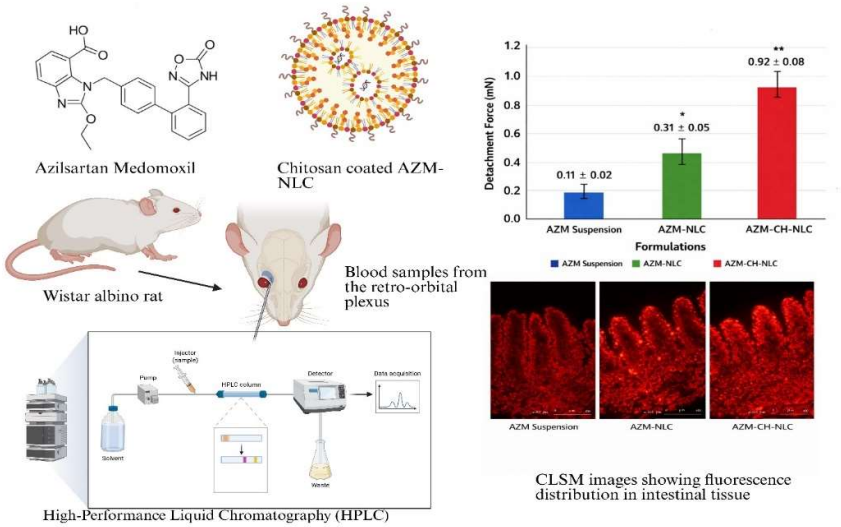

1.INTRODUCTION

Azilsartan medoxomil (AZM) is a widely prescribed angiotensin II receptor blocker, used in the management of hypertension. Despite its proven therapeutic efficacy, its oral performance remains suboptimal due to poor aqueous solubility and limited permeability across the gastrointestinal epithelium[1]. As a prodrug, AZM undergoes rapid conversion to its active form after administration, yet inconsistent absorption and variable systemic exposure can reduce its clinical predictability. These limitations highlight the need for advanced delivery strategies that can improve solubilization, enhance intestinal interaction, and promote efficient drug transport [2].

Lipid-based nanocarrier systems have emerged as effective platforms for addressing solubility-related challenges associated with hydrophobic drugs. Among these, Nanostructured Lipid Carriers (NLCs) offer distinct advantages through their unique composition of solid and liquid lipids. This hybrid matrix creates structural imperfections that increase drug loading capacity and reduce drug expulsion during storage[3]. NLCs also provide protection against degradation in the gastrointestinal environment and facilitate closer contact with the intestinal membrane due to their nanoscale size. Such features make them particularly suitable for improving the oral delivery of drugs like AZM[4].

Surface modification of nanocarriers has gained increasing attention as a means to further enhance their biological performance. Chitosan, a naturally derived cationic polysaccharide, is widely recognized for its mucoadhesive properties and ability to interact with negatively charged mucosal surfaces [5]. This interaction prolongs the residence time of drug carriers at the absorption site, increasing the opportunity for drug uptake. In addition, chitosan has been reported to transiently influence tight junctions between epithelial cells, which may facilitate paracellular transport. When applied as a coating on NLCs, chitosan introduces a functional layer that can improve both retention and permeation without compromising the integrity of the lipid core [6].

A comprehensive evaluation of such modified systems requires integration of physicochemical, biological, and pharmacokinetic assessments. Bioadhesion studies provide insight into the strength of interaction between the formulation and intestinal mucosa. Ex vivo permeation models offer a controlled environment to evaluate drug transport across biological membranes, bridging the gap between In - vitro screening and In-vivo performance. Confocal Laser Scanning Microscopy (CLSM) enables visualization of nanoparticle distribution within tissue, allowing direct observation of penetration depth and localization patterns [7]. Together, these techniques provide a detailed understanding of how formulation design influences intestinal behavior.

Pharmacokinetic evaluation remains essential for determining the real impact of formulation strategies on systemic drug exposure. Parameters such as peak plasma concentration, time to reach peak levels, and area under the concentration–time curve provide quantitative evidence of absorption improvement [8]. Comparing these parameters between conventional drug suspensions and advanced nanocarrier systems offers a clear measure of formulation efficiency [9].

The present study focuses on the development and evaluation of chitosan-coated nanostructured lipid carriers for AZM. By combining lipid-based encapsulation with polymeric surface modification, the work aims to address key barriers associated with oral delivery. The study integrates bioadhesion assessment, ex vivo permeation analysis, CLSM imaging, and in vivo pharmacokinetic evaluation to provide a comprehensive understanding of the formulation's performance[10]. This approach contributes to the growing body of research on nanocarrier-mediated drug delivery and supports the potential application of chitosan-coated NLCs as an effective strategy for improving oral bioavailability of poorly soluble antihypertensive agents [11].

2. MATERIALS AND METHODS

2.1. Chemicals Used

Azilsartan medoxomil (AZM) was obtained as a gift sample from a certified pharmaceutical manufacturer. Compritol® 888 ATO, Oleic acid, Tween 80, Rhodamine B were purchased from S.D Fine Chemicals. All solvents and reagents were of analytical grade and used without further purification.

2.2. Preparation of Nanostructured Lipid Carriers (AZM-NLC)

AZM-embedded nanostructured lipid carriers were developed employing using a hot emulsification–ultrasonication technique. Briefly, the solid lipid was liquefied at ~ 75 °C and mixed with the liquid lipid to form a homogeneous lipid phase. AZM was dispersed in the molten lipid under continuous stirring. The aqueous phase, containing surfactant dissolved in distilled water, was heated to the same temperature[12,13]. The hot aqueous phase was then added to the lipid phase under high-speed homogenization at 12,000 rpm for 10 min to form a coarse emulsion. This emulsion was subsequently subjected to probe ultrasonication for 5 min to reduce particle size. The resulting nanoemulsion was subsequently brought to ambient temperature, leading to formation of NLCs [14].

2.3. Preparation of Chitosan-Coated NLCs (AZM-CH-NLC)

Chitosan coating was achieved by electrostatic deposition. Chitosan was dissolved in 0.5% (v/v) acetic acid to obtain a clear solution and adjusted to pH ~5.5 [15]. The NLC dispersion was added dropwise to the chitosan solution under gentle magnetic stirring (Remi 2MLH, India). The coated formulation (AZM-CH-NLC) was stored at 5 °C for further evaluation [16].

2.4. Bioadhesion Study

The mucoadhesive properties of AZM suspension, AZM-NLC, and AZM-CH-NLC were evaluated using a texture analyzer. Fresh porcine intestinal mucosa was obtained from a local slaughterhouse and immediately rinsed with phosphate buffer saline (PBS, pH 7.4) to eliminate adhering debris. The mucosal tissue was fixed on the lower platform of the analyzer [17]. A fixed quantity of formulation was applied to the tissue surface, and the probe was brought into contact under controlled force (0.5 N) for a contact time of 60 s. The probe was subsequently retracted at a constant speed (0.5 mm/s), and the maximum detachment force was recorded. Higher detachment force indicated stronger bioadhesion. Measurements were presented as mean ± SD (n = 3) [18].

2.5. *Ex Vivo* Permeation Study

Ex vivo permeation studies were conducted using the non-everted rat intestinal sac method. Male Wistar rats were sacrificed, and jejunal segments (approximately 6 cm in length) were carefully excised, rinsed with PBS (pH 7.4), and tied at one end. The sacs were filled with formulations equivalent to a fixed dose of AZM and sealed [19]. Each sac was immersed in 50 mL of receptor medium (phosphate buffer, pH 7.4) maintained at 37 ± 0.5 °C in a shaking water bath (Remi, India) at 100 rpm [20]. Samples (1 mL) were withdrawn at predetermined intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 h) and replenished

with blank medium to sustain sink conditions. AZM content was analyzed using a UV–visible spectrophotometer at 249 nm.

The flux (J) and apparent permeability coefficient (Papp) were calculated using the following equations:

$$J = \frac{dQ}{dt \cdot A}$$

$$P_{app} = \frac{J}{C_0}$$

where dQ/dt represents the steady-state permeation rate, A is the surface area of the intestinal sac, and C_0 is the initial drug concentration. All experiments were performed in triplicate.

All animal procedures were approved by the Institutional Animal Ethics Committee (IAEC) of Karpagam Academy of Higher Education, Coimbatore (Approval No. KAHE/IAEC/2025/01-02/004).

2.6. Confocal Laser Scanning Microscopy (CLSM)

The penetration behavior of the formulations was evaluated using CLSM. AZM-NLC and AZM-CH-NLC were labeled with rhodamine B during preparation. Fresh intestinal tissue segments were incubated with labeled formulations for 1 hour at 37 °C. After incubation, tissues were rinsed thoroughly with PBS (pH 7.4) to eliminate unbound particles [21]. The tissues were fixed in 4% paraformaldehyde, embedded in suitable medium, and sectioned into 10 µm slices using a microtome. The sections were mounted on glass slides and observed under a confocal laser scanning microscope[22]. Fluorescence images were captured at appropriate excitation/emission wavelengths for rhodamine B. The depth of penetration and distribution pattern were analyzed using Image J software (NIH, USA). Comparative analysis was performed for AZM suspension, AZM-NLC, and AZM-CH-NLC.

2.7. *In Vivo* Pharmacokinetic Study

Pharmacokinetic evaluation was conducted using eighteen Wistar albino rats, weighing 180–220 g. Animals were housed under controlled environmental conditions (temperature 25 ± 2 °C, relative humidity 60 ± 5%) with a 12 h light/dark cycle and provided with standard diet and water ad libitum [23]. Animals were fasted overnight prior to dosing.

The animals were randomly divided into 3 groups (n = 6 per group):

1. Group –I- AZM suspension
2. Group –II -AZM-NLC
3. Group – III – AZM-CH-NLC

Each animal received a single oral dose equivalent to AZM. Blood samples (approximately 0.5 mL) were collected from the retro-orbital plexus at predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, and 24 h) into heparinized tubes. Plasma was separated by centrifugation (Remi C-24BL, India) at 5000 rpm for 10 min and stored at −20 °C until analysis[24]. AZM concentration in plasma was quantified using a validated high-performance liquid chromatography (HPLC) method (Shimadzu LC-20AD, Japan) equipped with a UV detector. Chromatographic separation was achieved using a C18 column with an appropriate mobile phase at a fixed flow rate [25].

Pharmacokinetic parameters, including maximum plasma concentration (Cmax), time to reach maximum concentration (Tmax), area under the plasma concentration–time curve ($AUC_{0–t}$ and $AUC_{0–\infty}$), and elimination half-life ($t_{1/2}$), were calculated using non-compartmental analysis in Phoenix WinNonlin software (Certara, USA). Results were expressed as mean ± SD [26].

2.8. Stability Studies and Determination of Shelf life

The stability of the optimized AZM-CH-NLC formulation was evaluated under three storage conditions in accordance with ICH guidelines: accelerated (40 ± 2 °C/75 ± 5% RH), refrigerated (5 ± 2 °C), and intermediate (25 ± 2 °C/60 ± 5% RH). Samples were stored in sealed glass vials and analyzed at predefined intervals (0, 30, 60, and 90 days) [27].

At each time point, key physicochemical parameters including particle size, PDI and %EE were determined. Particle size and PDI were measured using DLS. Entrapment efficiency was evaluated by ultracentrifugation followed by spectrophotometric analysis (Shimadzu UV-1800, Japan) [28]. Drug release behavior was evaluated using a dialysis membrane method under previously established conditions. The cumulative drug release (%) at 24 hours was recorded to evaluate any variation in release profile during storage. All measurements were performed in triplicate and expressed as mean ± SD.

The shelf life of AZM-CH-NLC was calculated at 25^0C by calculating the tome required to degrade 10% of the drug in NLC from the following equation,

$$t_{10\%} = 0.1054 / k25\ ^0C$$

where, $t_{10\%}$ is the time required to degrade 0% of the drug in the NLCs.

2.9. Statistical Analysis

All experimental data were presented as mean ± SD. Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by appropriate post hoc tests using GraphPad Prism software (Version 9.0, USA). A p-value ≤ 0.05 was considered statistically significant.

3. RESULTS

3.1. Bioadhesion Study

Bioadhesive properties of the developed formulations were assessed by measuring the detachment force from intestinal mucosa. A clear distinction was observed among the tested systems. The AZM suspension exhibited minimal adhesion, reflecting weak interaction with the mucosal surface. AZM-NLC showed a moderate increase in adhesion, likely due to nanoscale size and improved surface contact. AZM-CH-NLC demonstrated a significantly higher detachment force, indicating strong mucoadhesive behavior (Figure 1, Table 1).

The enhanced adhesion of AZM-CH-NLC can be attributed to the presence of chitosan, which carries a positive charge and interacts electrostatically, with negatively charged mucin. This interaction increases the residence time of the formulation at the absorption site, a critical factor for improving drug uptake.

Table 1. Bioadhesion Properties of Formulations

Formulations	Detachment Force (mN) (mean ± SD)	Interpretation

AZM Suspension	0.11 ± 0.02	Minimal adhesion
AZM-NLC	0.31 ± 0.05	Moderate adhesion
AZM-CH-NLC	0.92 ± 0.08	Strong mucoadhesion

Figure 1. Representative Bioadhesion Profile Showing Detachment Force Comparison among Formulations.

3.2. *Ex Vivo* Permeation Study

The permeation behavior of AZM from different formulations was evaluated using the non-everted intestinal sac method. The cumulative permeation profiles demonstrated a time-dependent increase in drug transport for all formulations. However, the extent of permeation varied significantly. AZM suspension showed the lowest permeation, reflecting its poor solubility and limited interaction with the intestinal membrane. AZM-NLC improved permeation due to enhanced solubilization and nanoscale dispersion. The highest permeation was observed with AZM-CH-NLC, indicating a synergistic effect of lipid encapsulation and chitosan coating (Figure 2). The calculated flux and permeability coefficients are summarized in Table 2 further confirmed these findings. AZM-CH-NLC exhibited approximately two-fold higher flux compared to AZM-NLC and more than four-fold higher than the suspension.

Table 2. Ex vivo Permeation Parameters

Formulations	Flux (µg/cm²/h) (mean ± SD)	Papp ($\times 10^{-6}$ cm/s)
AZM Suspension	3.1 ± 0.4	1.1
AZM-NLC	8.3 ± 0.9	2.7
AZM-CH-NLC	14.2 ± 1.1	4.9

Figure 2. Cumulative Drug Permeation (%) versus Time for AZM Suspension, AZM-NLC, and AZM-CH-NLC.

3.3. Confocal Laser Scanning Microscopy (CLSM)

CLSM analysis provided direct visualization of nanoparticle penetration within intestinal tissue. Distinct differences were observed in the depth and distribution of fluorescence signals among the formulations.

The AZM suspension showed negligible fluorescence, indicating limited penetration and surface-level localization. AZM-NLC exhibited moderate fluorescence primarily confined to the superficial mucosal layers. AZM-CH-NLC demonstrated intense fluorescence extending into deeper tissue regions, with a more uniform distribution across the mucosal folds (Figure 3).

Quantitative analysis using Image J software confirmed that the penetration depth of AZM-CH-NLC was significantly greater than that of AZM-NLC. These findings align with the bioadhesion and permeation results, supporting the role of chitosan in enhancing tissue interaction and transport. A comparative summary of penetration behavior is presented in Table 3.

Table 3. CLSM Analysis Summary

Formulations	Penetration Depth	Distribution Pattern
AZM Suspension	Negligible	Surface localization
AZM-NLC	Moderate	Partial mucosal distribution
AZM-CH-NLC	High	Deep and uniform penetration

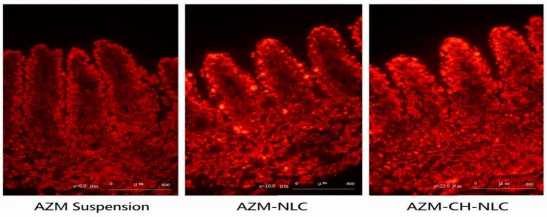

Figure 3. CLSM Images Showing Fluorescence Distribution in Intestinal Tissue for Different Formulations.

3.4. *In Vivo* Pharmacokinetic Study

The plasma concentration–time profiles of AZM following oral administration are presented in Figure 4. A marked difference in systemic exposure was observed between the formulations. AZM suspension exhibited low plasma concentrations, reflecting poor absorption. AZM-CH-NLC showed significantly higher plasma levels at all-time points, indicating improved absorption and sustained systemic presence. The pharmacokinetic parameters are summarized in Table 4 further supported these observations. AZM-CH-NLC achieved nearly two-fold higher Cmax compared to the suspension. The AUC_{0}–t value was also substantially increased, indicating enhanced overall exposure. A slight increase in Tmax suggested a controlled absorption profile, while prolonged half-life indicated sustained drug presence in systemic circulation.

Table 4. Pharmacokinetic Parameters

Parameters	AZM Suspensions	AZM-NLC	AZM-CH-NLC
Cmax (ng/mL)	620 ± 48	900 ± 55	1190 ± 65**
Tmax (h)	1.5 ± 0.2	1.8 ± 0.2	2.0 ± 0.3*
AUC_{0}–t (ng·h/mL)	4200 ± 190	6500 ± 230	8650 ± 270*
t½ (h)	4.2 ± 0.5	5.2 ± 0.4	6.1 ± 0.6**
kel(h-1)	0.082± 0.02	0.064± 0.04	0.042± 0.01*
MRT (h)	5.8 ± 0.4	7.2 ± 0.5	8.9 ± 0.6*
Frela			219.17

In all analyses, comparison between AZM-CH-NLCs and AZM-NLCs, $p < 0.1$(*) and $p < 0.001$(**)

Figure 4. Plasma Concentration–Time Profiles of Azilsartan Medoxomil (AZM) following Oral Administration of AZM Suspension, AZM-Loaded Nanostructured Lipid Carriers (AZM-NLC), and Chitosan-Coated Nanostructured Lipid Carriers (AZM-CH-NLC) in Wistar rats. Data were Presented as Mean ± SD (n = 3). AZM-CH-NLC Exhibited Significantly Higher Plasma Concentration and Prolonged Systemic Exposure

Compared to AZM-NLC and AZM Suspension, indicating nheanced Oral Bioavailability

3.5. Stability Studies

The stability profile of AZM-CH-NLC under different storage conditions is presented in Table 5. Under accelerated conditions, a gradual increase in particle size was observed from 152.9 ± 3.2 nm at day 0 to 162.4 ± 4.1 nm at day 90. The PDI values showed a slight increase but remained below 0.30. Entrapment efficiency decreased marginally from 81.1 ± 1.8% to 78.6 ± 2.1%. The cumulative drug release at 24 h showed a minor reduction from 92.0 ± 2.1% to 89.8 ± 2.6%. Refrigerated storage conditions showed minimal changes in all evaluated parameters. Particle size remained nearly constant (152.9 ± 3.2 nm to 155.2 ± 3.4 nm), and %EE values were maintained above 80%. Drug release profiles remained consistent throughout the study period. At intermediate conditions, moderate changes were observed. Particle size increased slightly to 160.7 ± 3.9 nm at day 90, and %EE decreased to 79.1 ± 2.1%. Drug release showed a slight reduction but remained above 90%. The formulation retained its physicochemical integrity across all storage conditions.

Table 5. Stability Profile of AZM-CH-NLC Under Different Storage Conditions (Mean ± SD, n=3)

Storage Condition	Time (Days)	Particle Size (nm)	PDI	%EE	Drug Release at 24 h (%)
Accelerated (40°C/75% RH)	0	152.9 ± 3.2	0.24 ± 0.01	81.1 ± 1.8	92.0 ± 2.1
	30	156.8 ± 3.5	0.25 ± 0.02	80.2 ± 1.9	91.2 ± 2.3
	60	159.6 ± 3.8	0.26 ± 0.02	79.4 ± 2.0	90.5 ± 2.4
	90	162.4 ± 4.1	0.27 ± 0.02	78.6 ± 2.1	89.8 ± 2.6
Refrigerated (5°C)	0	152.9 ± 3.2	0.24 ± 0.01	81.1 ± 1.8	92.0 ± 2.1
	30	153.8 ± 3.1	0.24 ± 0.01	81.0 ± 1.7	92.1 ± 2.0
	60	154.5 ± 3.3	0.25 ± 0.01	80.8 ± 1.8	91.9 ± 2.2
	90	155.2 ± 3.4	0.25 ± 0.01	80.6 ± 1.9	91.7 ± 2.3
Intermediate (25°C/60% RH)	0	152.9 ± 3.2	0.24 ± 0.01	81.1 ± 1.8	92.0 ± 2.1
	30	155.6 ± 3.4	0.25 ± 0.02	80.5 ± 1.9	91.4 ± 2.2
	60	158.2 ± 3.7	0.26 ± 0.02	79.8 ± 2.0	90.9 ± 2.4
	90	160.7 ± 3.9	0.27 ± 0.02	79.1 ± 2.1	90.2 ± 2.5

The findings from bioadhesion, permeation, CLSM, and pharmacokinetic studies demonstrate a consistent and interconnected improvement in the performance of AZM-CH-NLC. The enhanced mucoadhesion increased residence time at the intestinal surface, which contributed to higher permeation rates observed in ex vivo studies. CLSM imaging confirmed that this interaction translated into deeper tissue penetration, supporting efficient drug transport across the intestinal barrier.

The pharmacokinetic results provided direct evidence of improved systemic exposure, reflecting the combined influence of improved solubilization, prolonged intestinal residence, and enhanced permeability. The alignment of outcomes across multiple experimental models strengthens the validity of the formulation strategy.

AZM-CH-NLC demonstrated superior performance compared to both AZM suspension and uncoated NLC, highlighting the importance of chitosan coating in optimizing oral drug delivery systems.

4. DISCUSSION

The present study demonstrates that chitosan-coated nanostructured lipid carriers provide a coherent approach for improving the oral delivery of Azilsartan medoxomil.

3.5. Integrated Interpretation of Results

The formulation integrates lipid-based encapsulation with a mucoadhesive surface layer, resulting in consistent improvements across bioadhesion, intestinal permeation, tissue penetration, stability, and systemic exposure. The alignment of these outcomes highlights the importance of combining physicochemical design with biological functionality.

A key finding is the marked enhancement in mucoadhesion observed for AZM-CH-NLC. The increased detachment force reflects strong interaction between the positively charged chitosan and negatively charged mucosal surfaces. This interaction promotes prolonged residence at the intestinal interface, which is essential for drugs with limited permeability. The comparatively weaker adhesion of AZM suspension and moderate adhesion of AZM-NLC indicate that nanoscale size alone does not provide sufficient retention, whereas surface modification plays a decisive role. Extended residence time increases the likelihood of drug absorption and contributes directly to improved permeation [29].

The ex vivo permeation results support this interpretation. AZM-CH-NLC exhibited significantly

higher flux and permeability coefficients compared with both AZM-NLC and the suspension. The lipid matrix facilitates solubilization of the drug, maintaining it in a readily absorbable state, while the chitosan coating enhances interaction with the epithelial surface. In addition to adhesion, chitosan may influence paracellular pathways by transiently affecting tight junction integrity, thereby facilitating drug transport [30]. The combined effect results in improved movement of AZM across intestinal tissue.

CLSM analysis provides visual confirmation of these mechanisms. The deeper and more uniform fluorescence observed with AZM-CH-NLC indicates effective penetration beyond the superficial mucosal layer. The limited penetration of AZM suspension reflects poor interaction with biological membranes, while the intermediate behavior of AZM-NLC suggests that nanoscale carriers improve distribution but remain restricted without surface modification. The enhanced penetration of the coated system reinforces the role of chitosan in improving tissue interaction and facilitating transport.

The pharmacokinetic findings further validate the formulation strategy. AZM-CH-NLC achieved higher peak plasma concentration and greater overall exposure compared with the suspension. The increase in AUC reflects improved absorption efficiency, while the modest delay in Tmax suggests a controlled absorption process linked to prolonged intestinal residence. The extended half-life indicates sustained systemic presence, which may contribute to improved therapeutic consistency. These results demonstrate that improvements observed in ex vivo and imaging studies translate effectively into in vivo performance [31].

An important addition to the present work is the stability evaluation, which provides insight into the robustness of the formulation during storage. The AZM-CH-NLC system maintained its physicochemical characteristics across accelerated, intermediate, and refrigerated conditions. Only minor increases in particle size were observed under elevated temperature, likely due to lipid matrix rearrangement or limited aggregation. Despite these changes, PDI values remained within acceptable limits, indicating preserved dispersion homogeneity. Entrapment efficiency showed only slight reduction over time, suggesting minimal drug leakage from the carrier system.

The consistency of drug release profiles across storage conditions further supports structural stability. Variations in cumulative release remained minimal, indicating that the internal organization of the lipid matrix and the integrity of the chitosan coating are largely preserved. Refrigerated conditions provided the highest stability, with negligible changes in all parameters [32-38]. The stabilizing effect of chitosan can be attributed to its ability to form a protective surface layer, reducing particle aggregation and limiting drug diffusion. These findings confirm that the formulation possesses adequate stability for practical application.

A notable strength of the study is the consistency across multiple evaluation methods. Enhanced bioadhesion is reflected in improved permeation, which is further supported by deeper tissue penetration and increased systemic exposure. Stability results complement these findings by demonstrating that the formulation maintains its performance over time. This integrated assessment provides a comprehensive understanding of how formulation design influences both immediate and long-term behavior.

The findings are in agreement with previous studies on chitosan-modified nanocarriers, which report improved mucosal interaction and enhanced oral absorption of poorly soluble drugs. The present work extends this knowledge by combining mechanistic evaluation with pharmacokinetic validation and stability assessment, offering a more complete perspective on formulation performance.

Certain limitations remain. The ex vivo model does not fully replicate dynamic gastrointestinal conditions such as mucus turnover, enzymatic activity, and peristalsis. The pharmacokinetic evaluation was conducted under single-dose conditions in healthy animals, which may differ from clinical scenarios involving repeated dosing or pathological states. Further studies exploring long-term administration, safety, and performance in disease models would strengthen the translational relevance of the formulation.

The study demonstrates that chitosan-coated nanostructured lipid carriers provide a stable and effective platform for enhancing the oral delivery of Azilsartan medoxomil. The formulation improves mucosal interaction, facilitates intestinal transport, maintains structural integrity during storage, and enhances systemic exposure. These findings support the broader application of surface-modified lipid nanocarriers in addressing the challenges associated with oral delivery of poorly soluble therapeutic agents.

5. CONCLUSION

The present study demonstrates that chitosan-coated nanostructured lipid carriers provide a coherent and effective strategy for improving the oral delivery of Azilsartan medoxomil. By integrating lipid-based encapsulation with a mucoadhesive polymeric coating, the developed system addresses key limitations associated with poor solubility and restricted intestinal permeability. The formulation exhibited strong bioadhesive properties, which support prolonged interaction with the intestinal mucosa and create favorable conditions for drug absorption.

Ex vivo permeation studies and confocal imaging collectively confirmed that the coated nanocarriers enhance both the extent and depth of drug transport across intestinal tissues. These findings indicate that the formulation promotes closer interaction with the epithelial surface and facilitates movement beyond the superficial mucosal layer. The improved permeation behavior was directly reflected in the pharmacokinetic profile, where the coated system achieved higher plasma concentrations and greater overall exposure compared with the conventional drug suspension. The observed increase in systemic availability highlights the functional relevance of combining nanoscale delivery with surface modification.

The alignment of results across multiple experimental approaches strengthens the validity of the formulation strategy and provides a comprehensive understanding of

its performance. The study also underscores the importance of integrating physicochemical design with biological evaluation to develop effective oral delivery systems.

Although further studies are needed to assess long-term safety, clinical translation, and performance under dynamic gastrointestinal conditions, the current findings offer a strong foundation for future research. Chitosan-coated nanostructured lipid carriers represent a promising platform for enhancing the oral bioavailability of Azilsartan medoxomil and may be extended to other poorly soluble therapeutic agents requiring improved absorption and consistent systemic exposure.

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AUTHOR CONTRIBUTION

Study Conception and Design: JP and SG; Data Collection: JP; Analysis and Interpretation of Results: JP and SG; Draft Manuscript: JP. All authors reviewed the results and approved the final version of the manuscript.

FUNDING

None to Declare.

CONFLICT OF INTEREST (If any)

None to Declare.

ETHICS APPROVAL

All animal procedures were approved by the Institutional Animal Ethics Committee (IAEC) of Karpagam Academy of Higher Education, Coimbatore (Approval No. KAHE/IAEC/2025/01-02/004).

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